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Investigation of oxidative stress and Interleukin 33 (IL33) level in pericardial fluid of patients with coronary artery bypass surgery

Ömer Göç[1], Mustafa Göz[2], Reşat Dikme[1], Mahmut Padak[1]
[1] Department of Perfusion Techniques, Vocational School of Health Services, Harran University, Şanlıurfa
[2] Department of Cardiovascular Surgery, Private Anadolu Hospital, Bursa, Turkey

Abstract

Aim: Pericardial fluid composition changes in coronary artery disease. This study aims to investigate interleukin 33 (IL 33), total antioxidant status (TAS), total oxidative stress (TOS), and oxidative stress index (OSI) levels in the pericardial fluid and blood plasma of patients who have undergone coronary artery bypass surgery and contribute to the understanding of the pathophysiology of the disease.

Material and Methods: In the study, IL 33, TAS, TOS, and OSI levels were determined in the pericardial fluid and blood plasma of 40 patients who had undergone coronary artery bypass surgery, and the relationship between these parameters was investigated.

Results: IL 33 level in pericardial fluid (51.44 pq/mL) was found to be higher than Plasma IL 33 level (32.31 pq/mL). A significant positive correlation was found between OSI and TOS in pericardial fluid and plasma ($p<0.01$). A significant negative correlation was found between OSI and TAS in pericardial fluid ($p<0.01$).

Discussion: IL 33 level was found to be low in patients with coronary artery disease. A higher IL 33 level in pericardial fluid indicates that IL 33 is specific to the heart tissue and passes from the heart tissue to the pericardial space. TOS caused by cellular stress during the inflammation of the coronary arteries directly triggered OSI. IL 33 level may be increased to prevent damage to cells due to TOS and OSI. This study shows us that pericardial fluid can reflect physiological and biochemical changes in the heart, and therefore pericardial fluid can be used for diagnostic and therapeutic purposes.

Keywords

Cardiopulmonary Bypass, Coronary Artery Disease, Interleukin 33, Oxidative Stress, Pericardial Fluid

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Corresponding Author: Reşat Dikme, Department of Perfusion Techniques, Vocational School of Health Services, Harran University, Yenişehir Campus, Haliliye, 63300, Şanlıurfa, Turkey.
E-mail: rdikme@harran.edu.tr P: +90 535 458 78 44 F: +90 414 318 32 09
Corresponding Author ORCID ID: https://orcid.org/0000-0001-9157-7830
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Introduction

Recently, pericardial fluid (PF) can be used in addition to blood and heart tissue for the diagnosis of cardiovascular diseases (CVD). PF analysis provides an understanding of many pathophysiological mechanisms in various pericardial and CVD. In many studies conducted to compare cardiac biomarkers, better results were obtained from PF studies than blood plasma. The PF contains a variety of paracrine modulators, such as coronary vasomotor tone, coronary vasomotor tone, heart rate, blood pressure cardiomyocyte function and prostanoids, natriuretic peptides and endothelins [1]. Although the PF's protein concentration is lower than the plasma, the electrolyte content is considered to be an ultrafiltrate of this liquid blood plasma because it is close to the plasma [2].

CVD can be diagnosed by using various components released from the heart muscle to the circulation and pericardial cavity [2]. In the pathophysiology of CVD, important information about the disease can be obtained by examining the PF, which is the closest place to the heart tissue. For example, the presence of atrial natriuretic factor and brain natriuretic factor approximately 12 times higher in PF in heart failure suggests that these factors play a pathophysiological role as an autocrine or paracrine factor in heart failure [3]. Little is known about the immunological environment of PF, which has been studied in many ways [4, 5].

Interleukin 33 (IL 33) is a member of the IL-1 family that regulates the host response to infectious, inflammatory and immunological events; It is a bifunctional protein that acts as an intracellular nuclear factor with transcriptional properties and a proinflammatory cytokine.

Recently, serious studies have been started on IL 33 due to its effect on CVD [6]. IL 33 is released during cellular damage and stress; It has a cardioprotective effect by binding to a heterodimeric receptor complex consisting of ST2 and IL-1R accessory protein (IL-1RAP). Since the expression of IL 33 is intense in coronary artery endothelium, coronary artery smooth muscle cells, cardiomyocytes and cardiac fibroblasts, the role of IL 33 in various cardiovascular disorders should be investigated [7, 8].

IL 33 has a protective effect in conditions such as atherosclerosis, obesity, Type II diabetes and cardiac remodeling [9]. IL 33 decreases the development of atherosclerosis by increasing the production of oxidized-LDL antibodies stimulated by IL-5 [10]. In addition, it has been reported that IL 33, which increases the interaction of fibroblast and cardiomyocyte, has a beneficial role in heart failure [8]. Decreased expression of IL 33 with angiogenic or inflammatory stimuli indicates a possible role of IL 33 in endothelial cell activation and angiogenesis [11].

In a study in rats, treatment with IL 33 prevented hypertrophy in pressure-loading models of the ventricles. In hypoxia, IL 33 saved cardiomyocytes from apoptosis. Treatment with IL 33 in ischemia-reperfusion during myocardial infarction (MI) reduced the size of the infarct, corrected ventricular dilatation, suppressed caspase 3, and increased apoptosis inhibitors [12].

Despite the information available, data on IL 33 is currently insufficient. Although the presence of IL 33 has been investigated and detected in many living materials in many previous studies, its presence in PF has not been investigated. Since PF is difficult to obtain. For this reason, it is important to examine the presence of IL 33 in the PF of coronary artery patients undergoing open-heart surgery, where PF is easy to obtain. This research is important because the mechanism of increase-decrease of IL 33 is not known, there is no IL 33 study in PF and there are unanswered questions about the effect pathway of IL 33.

Material and Methods

Ethical committee approval

The present study was approved by the local ethics committee (Approval number: 25.01.2016-16/01/13).

Patients included in the study

Forty patients (28 males+12 females, mean age: 60.04 years) who underwent coronary artery bypass surgery with the cardiopulmonary bypass method were included in this study.

Obtaining blood and PF

Blood samples taken from the patients before surgery were transferred to glass tubes (10 ml, Vacutainers/BD Biosciences) with sterile and gel-free heparin (0.2 ml, Nevparin injectable 25000 IU/5 mL) and were transferred to the laboratory with ice packs. After the centrifugation step (5000 rpm, 5 min), the supernatant part of the blood (plasma) was taken into a sterile Eppendorf tube (1.5 mL, Eppendorf) and kept at -80oC until the study day. Median sternotomy was first performed to obtain PF from the patients. Then the pericardium was opened and the PF was aspirated with a sterile syringe. PF was then transferred to sterile glass tubes (10 mL, Vacutainers/BD Biosciences) and stored in an ice-filled container. The supernatant portion of the PF centrifuged at 4000 rpm (5 min) was transferred to a sterile Eppendorf tube (1.5 mL, Eppendorf) and stored at -80oC until the study day.

Quantification of IL 33 in plasma and PF with ELISA

For the detection of IL 33 in plasma and PF, the commercial kit Human IL 33 (Interleukin 33) (Elabscience, catalog number, E-EL-H2402 www.elabscience.com) ELISA Kit was used.

Measurement of Total Oxidant Status (TOS), Total Antioxidant Status (TAS) and Oxidative Stress Index (OSI) in Plasma and PF

TOS Level Measurement

TOS levels were determined using a new automatic measurement method developed by Erel [13]. Results were expressed as μmol H2O2 Equivalent/L.

TAS Level Measurement

TAS levels were determined using a new automatic measurement method developed by Erel [14]. Results are expressed as mmol Trolox Equivalent/L.

OSI Measurement

OSI, which is accepted as an indicator of Oxidative Stress, is expressed as a percentage of the ratio of TOS levels to TAS levels. While calculating OSI in the examples, TAS levels were multiplied by 10 and TOS levels and units were equalized. Results are expressed as Arbitrary Units (AU) [15, 16].

Statistical Analysis

Statistical analyzes were performed using the SPSS Version 25 (IBM, SPSS Inc. Chicago USA) computer program. The significance of the difference between the means of the groups was compared with the One-Way ANOVA test. The relationship between the parameters was investigated by Pearson's

correlation analysis. Values less than p < 0.05 were considered statistically significant.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

The minimum, maximum, mean and standard deviation results of the patients included in the study (28 males, 12 females, mean age: 60.04 Years), the number of coronary vessels, IL 33, TAS, TOS and OSI are shown in Table 1. The number of bypassed coronary arteries in the operated patients varied between 2 and 4. As a result of the statistical evaluation of the data obtained from the study, the correlation values between IL 33, TAS, TOS and OSI in the PF and Plasma of the patients are shown in Table 2. According to the statistical evaluation of the findings, a positive significant correlation (r= 0.978, p<0.01) was found between OSI and TOS in plasma. There was a negative significant correlation between OSI and TAS in PF (r= -0.509, p<0.01). A positive significant correlation (r= 0.722, p<0.01) was found between OSI and TOS in PF. Although there was a negative correlation between Pericardial IL 33 and Plasma IL 33 (r=-340), no statistically significant correlation was found between these two parameters (p=0.121, p>0.05) (Figure 1). As a result of the statistical evaluation of the data obtained from the study, the relationship between the number of coronary vessels bypassed during the operation and plasma and Pericardial IL 33 is shown in Table 3.

Table 1. Table of minimum, maximum, mean and standard deviation results between patients' age, number of coronary vessels, IL 33, TAS, TOS and OSI

	Minimum	Maximum	Mean	Std. Deviation
Number of coronary vessels	2	4	3,0455	0,78542
Plasma_TAS (mmol Trolox Equivalent/L)	1,54	2,27	1,7605	0,1568
Plasma_TOS (μmol H2O2 Equivalent/L)	8,6	32	12,5423	4,6999
Plasma_OSI (Arbitrary Units (AU))	0,51	1,76	0,7151	0,26003
Plasma_IL33 (pq/mL)	21	52,31	32,3164	11,98232
Pericard_TAS (mmol Trolox Equivalent/L)	0,9	1,68	1,3073	0,18846
Pericard_TOS (μmol H2O2 Equivalent/L)	5,7	19	14,4377	2,57942
Pericard_OSI (Arbitrary Units (AU))	0,38	1,67	1,1125	0,23345
Pericard_IL33 (pq/mL)	26,01	79,2	51,4418	18,34525

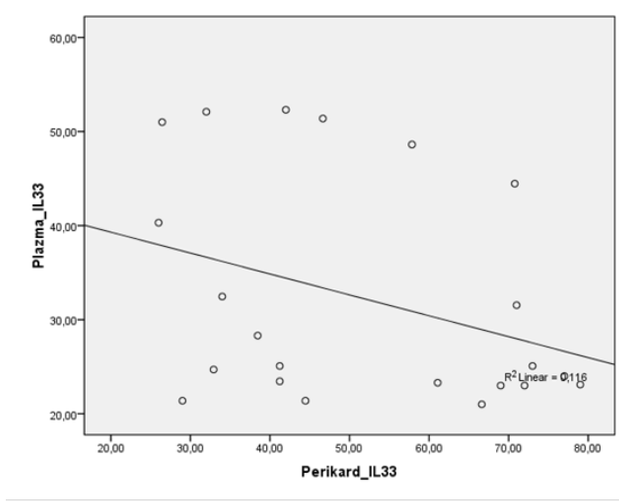

Figure 1. Correlation between Pericard IL 33 and Plasma IL 33 level

Table 2. Correlation chart between Pericard and Plasma IL 33, TAS, TOS and OSI

		Plasma TAS	Plasma TOS	Plasma OSI	Plasma IL33	Pericard TAS	Pericard TOS	Pericard OSI	Pericard IL33
Plasma TAS	r	1	0,089	-0,119	-0,085	0,228	0,124	-0,088	0,313
	p		0,695	0,597	0,706	0,308	0,583	0,698	0,156
Plasma TOS	r	0,089	1	,978**	-0,091	0,214	0,321	0,158	0,255
	p	0,695		0	0,688	0,34	0,145	0,483	0,252
Plasma OSI	r	-0,119	,978**	1	-0,079	0,173	0,302	0,18	0,192
	p	0,597	0		0,725	0,44	0,173	0,424	0,392
Plasma IL33	r	-0,085	-0,091	-0,079	1	0,069	-0,013	-0,248	-0,34
	p	0,706	0,688	0,725		0,761	0,953	0,266	0,121
Pericard TAS	r	0,228	0,214	0,173	0,069	1	0,042	-,509*	-0,132
	p	0,308	0,34	0,44	0,761		0,854	0,016	0,558
Pericard TOS	r	0,124	0,321	0,302	-0,013	0,042	1	,722**	0,21
	p	0,583	0,145	0,173	0,953	0,854		0	0,349
Pericard OSI	r	-0,088	0,158	0,18	-0,248	-,509*	,722**	1	0,364
	p	0,698	0,483	0,424	0,266	0,016	0		0,096
Pericard IL33	r	0,313	0,255	0,192	-0,34	-0,132	0,21	0,364	1
	p	0,156	0,252	0,392	0,121	0,558	0,349	0,096	

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

Table 3. Relationship between the number of bypassed coronary vessels and plasma and pericard IL 33

		Number of coronary vessels	Plasma_IL33	Pericard_IL33
Number of coronary vessels	r	1	0,309	-0,013
	p		0,173	0,956
Plasma_IL33	r	0,309	1	-0,386
	p	0,173		0,084
Pericard_IL33	r	-0,013	-0,386	1
	p	0,956	0,084	

(When the number of coronary bypass vessels performed and the plasma IL 33 (pq/mL) level were compared, although there was a positive correlation between the number of coronary vessel bypasses and plasma IL 33 (pq/mL) level, no significant correlation was found (r=0.309, p=0.956 >0.05) The number of coronary bypass vessels performed and Pericard IL 33 (pq/mL) level compared; There was a negative correlation between the number of coronary artery bypasses and Pericard IL 33 (pq/mL) level, but no significant correlation was found between these two parameters (r= -0.013, p=0.84 >0.05))

Discussion

In many previous studies, various angiogenic growth factors, cytokines, natriuretic peptides and classical vasoactive hormones were found in higher concentrations in PF compared to plasma [17]. In our study, IL 33, TOS and OSI levels in PF were found to be higher. TAS level was lower in PF. According to these data obtained, TOS caused by cellular stress during the inflammation of the coronary arteries directly triggered OSI, and the level of IL 33 may have increased in order to prevent damage to the cells due to TOS and OSI.

It has not been determined exactly in which diseases IL 33 is high or low and according to what. These observations suggest that IL 33 may have a complex regulatory role in CAD.

In a study conducted in patients with CAD, it was reported that IL 33 expression was high, circulating IL 33 levels were associated with the pathogenesis of atherosclerosis in patients with acute coronary syndrome and could be used as diagnostic indicators in terms of a new index value in these diseases [18]. In a study conducted in the Chinese Han population, the level of IL 33 in the plasma was found to be 233.67 pg/ml in coronary artery diseases [19]. In a study conducted on ischemic heart patients, serum IL 33 level was found to be 103.33 pg/ml in AMI patients, 157.60 pg/ml in stable angina patients, 122.21 pg/ml in unstable angina patients, and 61.85 pg/ml in control group patients [20]. In our study, however, the plasma IL 33 level was found to be lower (32.31 pg/ml). This shows us that the IL 33 level of patients who had coronary artery bypass surgery decreased.

In a study examining the relationship between oxidative stress and IL 33, it was found that IL 33 directly reduced oxidative stress [21]. In our study, it was determined that the IL 33 level increased in PF compared to plasma, against increased oxidative stress. This shows us that IL 33 level increases in case of oxidative stress.

In our study, IL 33, which is produced by many organs, should normally be found higher in plasma, but, surprisingly, it is found more in PF, which is in line with previous studies. The main factor in this situation may be the passage of IL 33 from the heart tissue to the PF. Perhaps IL 33 produced during cellular stress does not function in heart cells and may have leaked into the pericardial space. The high level of IL 33 in PF, which is the tissue closest to the heart, strengthens the acceptance of this fluid as an ultrafiltrate of blood plasma.

Although it is said that IL 33 protects the cell against stress, in fact, the good or bad aspects of high or low IL 33 need to be investigated. For example, cells with high IL 33 probably have a lot of cellular stress, so a high IL 33 actually indicates that that cell is under intense stress. Therefore, a high IL 33 may not mean good. A low IL 33 indicates less stress in the cell, which may actually be a good situation. In order to better evaluate the function of IL 33 in the cell, it would be better to study different cellular stress parameters with IL 33 in another study.

According to the results of the study, Plasma IL 33 level was high and PF IL 33 level was low in patients with a high number of bypassed coronary arteries.

Conclusion

This study showed that the IL 33 concentration of PF is different from the plasma and the IL 33 level is high in coronary artery patients. The reason why IL 33 is found more in PF may be the passage of IL 33 from the heart tissue to the pericardial space. In this study, it was also determined that IL 33 level increased against increasing oxidative stress and generated data about IL 33 level in PF in coronary artery patients. However, cellular-based studies are needed to examine the endogenous role of IL 33 and to determine the mechanism of increase and decrease. In the future, the effects of administration of IL 33 to the plasma or pericardial cavity should be investigated by animal experiments.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Conflict of interest

The authors declare no conflict of interest.

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